

*Cont
B
SUB
C
CON*

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF),

for prolonged time period sufficient to produce a culture having a compact multilayer like appearance whereby said culturing may be performed in the absence of a feeder layer;

- (iii) identifying EG cells contained therein.

B²
14. (Amended) An improved method of producing chimeric avians which comprises:

*SUB
C²*

- (i) isolating primordial germ cells from an avian;
- (ii) maintaining such PGCs in a tissue culture medium containing at least the following growth factors;

 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF) for a sufficient time to produce embryonic germ (EG) cells whereby said culture may be maintained in the absence of a feeder layer;

- (iii) transferring said EG cells into a recipient avian embryo; and
- (iv) selecting for chimeric avians which have the desired PGC phenotype.